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Stereoselective Access to Tubuphenylalanine and Tubuvaline: Improved Mn-Mediated Radical Additions and Assembly of A Tubulysin Tetrapeptide Analog

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Abstract

Synthesis of tubuphenylalanine and tubuvaline, α -substituted γ -amino acid building blocks for tubulysin family of antimetabolic compounds, has been improved using a radical addition reaction in the presence of unprotected hydroxyl functionality. The key carbon–carbon bond construction entails stereoselective Mn-mediated photolytic additions of alkyl iodides to the C=N bond of chiral *N*-acylhydrazones, and generates the chiral amines in high yield with complete stereocontrol. Reductive N–N bond cleavage and alcohol oxidation converted these amino alcohols into the corresponding γ -amino acids. The route to tubuvaline proceeded via peptide coupling with serine methyl ester, followed by a high-yielding sequence to convert the serine amide to a thiazole. Finally, peptide bond construction established the tubulysin framework in the form of a *C*-terminal alcohol analog. Attempted oxidation to the *C*-terminal carboxylate was unsuccessful; control experiments with dipeptide **18** showed a cyclization interfered with the desired oxidation process.

1. Introduction

The tubulysins are a group of unusual peptides distinguished by two novel γ -amino acid residues, tubuphenylalanine and tubuvaline, both of which are α,γ -disubstituted γ -amino acids. Initially isolated from culture broths of the myxobacteria *Archangium gephyra* and *Angiococcus disciformis*, these are extraordinary antimetabolic agents, with potency superior to vinblastine and dolastatin-10 (growth inhibition of human cervical carcinoma DSM ACC 158, tubulysin D: IC₅₀ 20 pg/mL).^{1,2} After further isolation efforts, the series now includes tubulysins A–I, U, and V, as well as pretubulysin (Scheme 1).^{3,4,5}

The extraordinary potency of tubulysins and their interesting impacts on tubulin biochemistry⁶ have inspired numerous efforts to probe their therapeutic potential through synthesis and medicinal chemistry. We disclosed our initial studies directed toward synthesis of the tubulysins in 2004,⁷ and the first total syntheses were achieved by Ellman (tubulysin D, 2006)⁸ and Zanda (tubulysins U and V, 2007).⁹ Despite numerous synthetic efforts (which have been reviewed^{10,11,12} and continue to emerge^{13,14,15}), accessing both of the γ -

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amino acid subunits tubovaline (Tuv, Scheme 1) and tubophenylalanine (Tup) with excellent stereocontrol has proven to be a difficult challenge. Prior tubulysin syntheses have been hampered by limited stereoselectivity in alkene or ketone reductions to generate the requisite configuration at the α carbon of the γ -amino acids. In general, most approaches to γ -amino acids are limited by reliance upon homologation of the naturally abundant α -amino acids. These factors emphasize the need for new and versatile methodology for γ -amino acid synthesis,^{16,17} and serving this need became the cornerstone of our synthetic strategy.

Our plan for a more versatile C–C bond construction approach to synthesis of γ -amino acids would obviate the limitations of naturally occurring α -amino acid precursors, facilitating preparation of γ -amino acids bearing unusual functionality or substitution patterns. Taking guidance from the tubulysins, we required that the ideal method would be applicable to strategic construction of either the C β –C γ or C γ –C δ bonds of γ -amino acids (as shown for Tup and Tuv, Scheme 1) and independent of substituents at the α -position. Stereoselective intermolecular additions of alkyl radicals to the C=N bonds are well-suited to this need; these reactions have been reviewed,^{18–26} and new ones continue to develop.^{27,28,29} We introduced a photolysis method^{30,31} for this reaction type which employs Mn₂(CO)₁₀ to generate alkyl radicals, leading to efficient additions of primary and secondary alkyl iodides to chiral *N*-acylhydrazones.^{32–35} The availability of efficient primary radical addition has opened many new synthetic applications; with earlier methods, primary alkyl radicals were prone to premature reduction or other side reactions (e.g., addition of ethyl radical under triethylborane or diethylzinc initiation).^{20–26} The functional group tolerance and non-basic conditions of this method show excellent potential for synthesis of multifunctional amines;^{36–41} the tubulysin γ -amino acids would test this potential. Indeed, our initial studies on tubulysins achieved preparation of Tuv precursor **A** and Tup itself (**B**) via these Mn-mediated radical addition reactions.⁷ Here we disclose improved preparations of **A** and **B** that demonstrate the functional group compatibility of a free hydroxyl during the Mn-mediated coupling, as well as tolerance of imino compounds that are prone to E1cb elimination. We further disclose a high-yielding sequence leading from **A** to tubovaline, and the assembly of a C-terminal alcohol analog of the tubulysin tetrapeptide.

2. Results and Discussion

Preparation of γ -Amino Acids

Improvements to our previously reported γ -amino acid syntheses began with an effort to address a low yield (56%) in the Mn-mediated coupling of phenylacetaldehyde hydrazone **4** (Scheme 2) with iodide **5a**, bearing a silyl-protected alcohol, en route to tubophenylalanine.⁷ To this end, we examined alternate iodide partners to find that iodide **5b**,⁴² bearing a free hydroxyl group, functioned more efficiently in the Mn-mediated radical addition. The reaction entailed photolysis (300 nm) of **4** and **5b** with Mn₂(CO)₁₀ in the presence of InCl₃ as a Lewis acid, and furnished 1,4-hydrazino alcohol **6b** in 79% yield, a significant improvement over the previously reported coupling. The identical stereochemical outcomes in **6a** and **6b** were correlated via *O*-silylation of **6b** in 98% yield, which established that the free hydroxyl group was not detrimental to stereoselectivity. Then, N–N bond cleavage afforded Tup precursor **7** with the C-terminus in the alcohol oxidation state.

Preparation of the γ -amino acid progenitor of tubuvaline followed the previously published route⁷ from known alcohol **8**⁴³ (Scheme 2). Jin's one-step Lemieux-Johnson oxidation⁴⁴ shortened this sequence by one step, but did not improve the overall yield. In the key step, the Mn-mediated coupling method was carried out with slow addition (10 h) of a solution of $\text{Mn}_2(\text{CO})_{10}$ in CH_2Cl_2 during the photolysis (254 nm, pyrex glassware). It should be noted that typical Mn-mediated radical addition reactions do not require 254 nm light nor slow addition for successful reaction, but in this case at least, it led to a small improvement in yield. Thus isopropyl iodide added to the C=N bond of hydrazone **9**, affording **10** in 84% isolated yield as a single diastereomer (versus 77% reported previously⁷). Treatment of the hydrazone **9** with fluoride ion (TBAF) resulted in loss of the benzyloxy substituent, but this E1cb process did not occur during the radical addition reaction, underscoring its non-basic character. In another improvement upon our previously reported route, modified conditions for N–N bond cleavage⁴⁵ furnished trifluoroacetamide **11** in 97% yield.

Elaboration of the Tubuvaline Thiazole—The thiazole portion of tubuvaline was envisioned to arise from serine via cyclization and oxidation. Desilylation and oxidation of **11** provided the γ -amino acid **A** (Scheme 3), to which serine methyl ester was attached in a peptide bond construction mediated by diethyl cyanophosphate (DECP). Silylation then afforded **12** in quantitative yield over the two steps. Alternatively, the serine could be installed with the silyl group already present, albeit with diminished coupling yield (83%). Next, conversion of the peptide bond to a thioamide was achieved with freshly prepared Belleau's reagent⁴⁶ added to a refluxing solution of **12** in THF; this afforded the thioamide **13** in dependably high yield. Desilylation of the serine hydroxyl group, then successive cyclization with diethylaminosulfur trifluoride (DAST)⁴⁷ and mild oxidation from thiazoline to thiazole with BrCCl_3 under Williams' conditions⁴⁸ completed the preparation of tubuvaline derivative **14** in excellent overall yield.

Assembly of the Peptide Backbone—Next, assembly of the peptide was initiated via saponification of tubuvaline methyl ester **14** with LiOH in aqueous MeOH (Scheme 4). This proceeded selectively in 98% yield, preserving the *N*-trifluoroacetyl protection in **15**. Meanwhile, $\text{Ba}(\text{OH})_2$ caused hydrolysis of the *N*-trifluoroacetamide of tubuphenylalaninol derivative **7**, releasing free amine **16**. The Tuv and Tup components then engaged in peptide coupling in the presence of DECP and Hunig's base, leading to dipeptide **17** in quantitative yield. Another hydrolytic deprotection with $\text{Ba}(\text{OH})_2$ exposed the primary amine at the *N*-terminus, where the final two amino acids, *N*-methylpipercolic acid (Mep) and isoleucine (Ile) would next be attached.

We explored several alternatives for coupling the Mep and Ile units to the *N*-terminus of **18**. One attractive approach was to introduce Ile as an azide, followed by hydrogenation of the azide with concomitant removal of the *O*-benzyl group in the Tuv fragment, with in situ coupling to an active ester form of Mep.⁸ This showed some promise, as the first coupling occurred smoothly (Equation 1). Unfortunately the azide reduction and coupling of **23** to Mep gave complex mixtures, due in part to incomplete removal of the *O*-benzyl group.

Coupling of **18** to a previously constructed Mep-Ile dipeptide⁹ emerged as a more reliable approach. The known dipeptide Mep-IleOH was prepared as its trifluoroacetate salt (**19**,

Scheme 4) via acylation of isoleucine *tert*-butyl ester with MepOH⁴⁹ and removal of the *tert*-butyl group with trifluoroacetic acid. An ethyl acetate solution of this salt was exposed to isobutyl chloroformate in the presence of *N*-methylpyrrolidine to form the mixed anhydride, and the Tuv-Tup dipeptide **18** was introduced. In several trials using this procedure, the tetrapeptide **20** was reliably obtained with yields ranging from 42–48%. Desilylation under typical conditions afforded primary alcohol **21** in quantitative yield.

To convert **21** to tubulylin V, all that remained was oxidation of the C-terminus to a carboxylic acid and debenzoylation of the C5' hydroxyl group. Toward this end, a number of oxidations were attempted, but useful oxidation products were not observed (neither aldehyde nor carboxylic acid). From examination of product mixtures in oxidations with PhI(OAc)₂ and TEMPO, mass spectra and ¹H NMR data suggested that the aldehyde was formed, then trapped intramolecularly by the γ -amido group. Although similar oxidations of related compounds have been reported,^{50,51} our attempts to purify a component of this material or make use of it for further transformations were unsuccessful. Toward a better understanding of the outcome, the simpler Tuv-Tup dipeptide **18** was used for a control experiment. After *N*-acylation with Boc₂O and desilylation, oxidation under the PhI(OAc)₂/TEMPO conditions gave cyclized hemiaminal **22** in 52% overall yield (Scheme 4). Although we were forced to consider an alternate path to access the tubulylins, this study placed the foundation for a modified route which is currently in progress.

3. Conclusion

In conclusion, a C-terminal alcohol analog in the tubulylin family has been assembled via a route which constructs both of the γ -amino acids with complete stereoselectivity (>98:2). The γ -amino acid synthesis expands the access to building blocks for bioorganic chemistry, including unusual peptide natural products^{52–58} and conformationally constrained peptide mimics.^{59,60} The key enabling methodology is a highly stereoselective Mn-mediated coupling of alkyl iodides with chiral *N*-acylhydrazones. Advances in this Mn-mediated radical addition chemistry attributed to this synthetic effort are (a) primary alkyl addition in the presence of unprotected hydroxyl functionality to access tubuphenylalanine in high yield, (b) avoidance of E1cb-type elimination of β -alkoxy functionality in a tubovaline precursor by employing non-basic isopropyl radical addition to the C=N bond, and (c) an alternative procedure involving slow addition of Mn₂(CO)₁₀ to improve the yield of **10**. Taken together with our previous Mn-mediated coupling studies,^{30,31,36–41} these advances broaden the scope and functional group compatibility of Mn-mediated radical additions to hydrazones, cementing the viability of this reaction for complex target-directed synthesis.

4. Experimental Section

Procedures and product characterization data for the Mn-mediated coupling steps are provided here; for complete experimental details, see Supporting Information.

Mn-Mediated Radical Addition: Hydrazino alcohol **6b**

A solution of hydrazone **4** (360 mg, 1.22 mmol) in CH₂Cl₂ (30 mL) was added to InCl₃ (541 mg, 2.45 mmol, dried under vacuum for ca. 12 h) in a pyrex Schlenk tube. The mixture was

stirred for 2 h at room temperature. Then iodide **5b** (degassed by bubbling argon for 15 min, 856 mg, 4.28 mmol) was added followed by addition of $\text{Mn}_2(\text{CO})_{10}$ (524 mg, 1.35 mmol) as a solid. The reaction mixture was degassed by bubbling argon for 15 min, then irradiated for 15 h using a Rayonet photochemical reactor (300 nm, pyrex glassware); the ambient temperature inside the irradiation chamber reached ca 35 °C. The reaction mixture was diluted with diethyl ether, then triethylamine (2.0 mL, 16 mmol) was added. After stirring 1 h, concentration and flash chromatography (petroleum ether/ethyl acetate, 3:1 → 1:2) afforded hydrazino alcohol **6b** (357 mg, 79% yield, dr >98:2) as a colorless oil; $[\alpha]_{\text{D}}^{24}$ -26.6; IR ν_{max} (NaCl, film) 3441, 2925, 1754, 1745, 1730, 1494, 1452, 1401, 1239, 1093, 1030 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 7.42–7.16 (m, 8H), 7.08–7.03 (m, 2H), 3.96–3.90 (m, 2H), 3.69–3.58 (m, 1H), 3.56–3.48 (m, 3H), 3.03 (dd, J = 13.4, 3.4 Hz, 1H), 2.80 (m, apparent d, J = 6.8 Hz, 2H), 2.45 (dd, J = 13.3, 10.1 Hz, 1H), 1.99–1.88 (m apparent octet, 1H), 1.58 (ddd, J = 14.3, 7.2, 5.5 Hz, 1H), 1.43 (ddd, J = 14.2, 6.5, 6.5 Hz, 1H), 0.93 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 158.5, 139.0, 135.6, 129.1, 129.1, 128.8, 128.5, 127.0, 126.4, 68.0, 65.7, 58.8, 58.5, 40.2, 37.3, 36.7, 32.5, 17.6; MS (ESI) m/e (rel. intensity) 391 ($[\text{M}+\text{Na}]^+$, 100), 369 ($[\text{M}+\text{H}]^+$, 57); Anal. Calcd for $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_3$: C, 71.71; H, 7.66; N, 7.60. Found: C, 71.06; H, 7.47; N, 7.48.

Mn-Mediated Radical Addition with Slow Addition: Hydrazine 10

To a solution of hydrazone **9** (300 mg, 0.62 mmol) in CH_2Cl_2 (31 mL) was added InCl_3 (275 mg, 1.24 mmol dried under vacuum for ca. 12 h), followed by isopropyl iodide (0.25 mL, 2.49 mmol, filtered through basic alumina). The mixture was stirred for 15 min at room temperature. Using a syringe pump, a solution of $\text{Mn}_2(\text{CO})_{10}$ in CH_2Cl_2 (10 mL) was added over 10 h at a rate of 1 mL/h while the mixture was irradiated using a Rayonet photochemical reactor (254 nm, pyrex glassware). After the addition was complete, irradiation was continued for another 5 h; the ambient temperature inside the irradiation chamber reached ca 35 °C. Concentration and flash chromatography (hexanes → 3:1 hexanes/EtOAc) afforded hydrazine **10** as a colorless oil (276 mg, 84% yield, dr >98:2). Analytical data for this material were consistent with the prior report.⁷

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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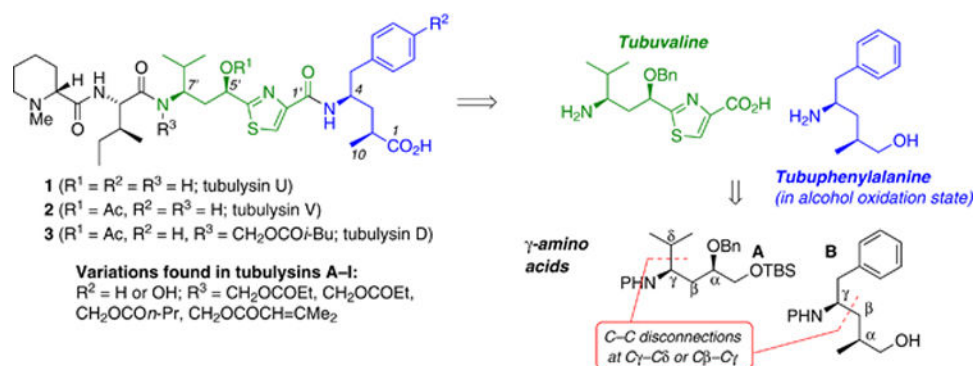
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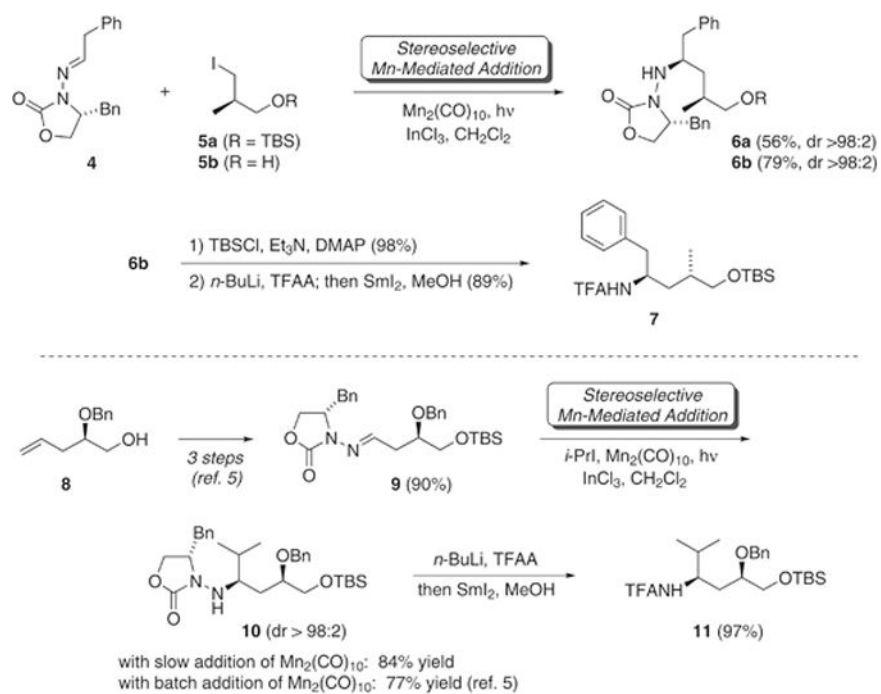
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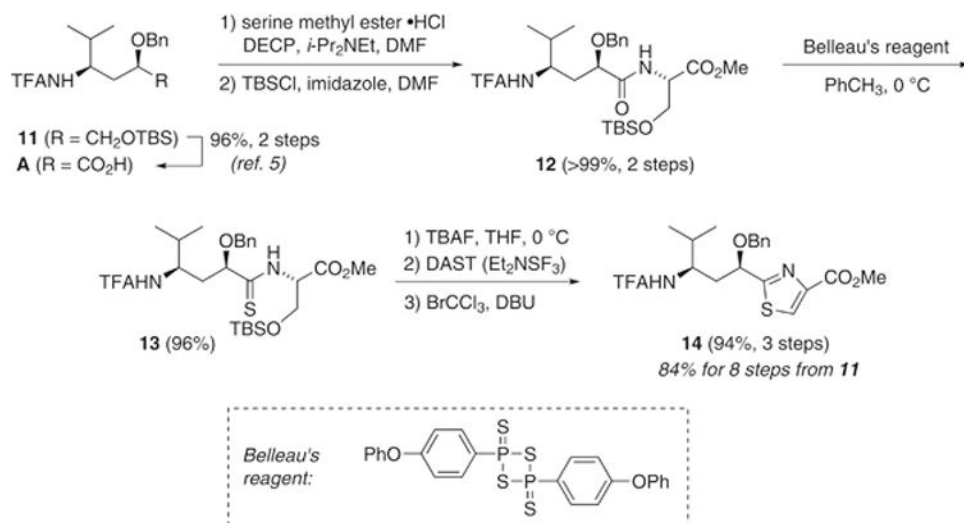
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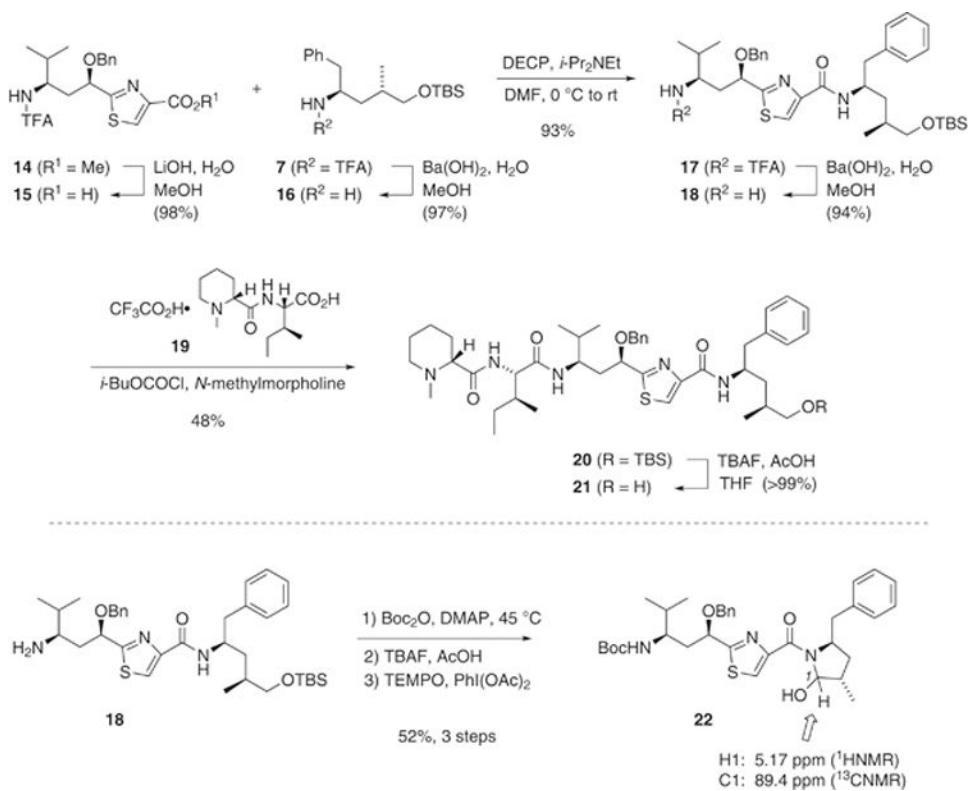
Scheme 1.
Retrosynthetic analysis of tubulysin, highlighting the γ -amino acids.



Scheme 2.
Mn-mediated radical additions in γ -amino acid synthesis.



Scheme 3.
Installation of the Tuv thiazole.



Scheme 4.
 Assembly of di- and tetrapeptides.